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Giuseppe Bogliani (*)

Population structure and loss of heterozygosity in relation to management in Sardinian semi-feral ponies

Abstract – Semi-feral horses which roam on the Giara di Gésturi under partial management were censused (513 individuals) and their population structure was compared with the daughter population living on Capo Caccia (27 individuals) where horses are left unmanaged. The sex ratio was close to 1 at Capo Caccia, but was female biased on the Giara (1 male per 8 females), where most yearling males are removed. The unbalanced sex ratio on the Giara greatly reduces the Effective Population Size and will cause a rapid loss of heterozygosity. In order to maintain a large amount of genetic variation the sex ratio on the Giara would have to be closer to 1.

Riassunto - Struttura della popolazione e perdita di variabilità genetica dovuta alla gestione dei cavallini sardi viventi allo stato semi-selvatico.

Sono stati censiti i cavallini della Giara di Gésturi (513 individui) sottoposti a manipolazioni da parte dell'uomo, e di Capo Caccia (27 individui), non manipolati in modo sistematico, e ne sono state comparate le strutture di popolazione. Il rapporto-sessi è prossimo a uno a Capo Caccia; è fortemente sbilanciato sulla Giara (1 maschio ogni 8 femmine), nella quale la maggior parte dei giovani maschi di un anno viene rimossa regolarmente. Il rapporto sessi sbilanciato sulla Giara riduce di molto le dimensioni della popolazione efficace (Effective Population Size) da utilizzarsi per le stime di perdita di eterozigosi in generazioni successive, e causerà una rapida perdita di variabilità genetica nel corso di poche generazioni. Per un efficace mantenimento del massimo di eterozigosi e della vitalità della popolazione nella Giara occorrerebbe portare il rapporto-sessi più vicino all'equilibrio.

Key words: *Equus caballus*, Population structure, Heterozygosity, Management, Sardinia.

(*) Dipartimento di Biologia Animale, Università di Pavia, Piazza Botta 9, 27100 Pavia, Italy.

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Introduction

The wild close relatives of some domesticated animals have become extinct in recent times, and thus the remaining genetic variation of the species can only be found in captive populations. However modern breeding techniques are oriented towards the selection of breeds that are well adapted to captivity and to economic goals. This is usually achieved by enhancing homozygosity for some characteristics, and in this way much genetic variation is being lost through artificial selection.

Such loss of genetic variability in domesticated animals will reduce the possibility of selecting breeds for characteristics which might be desirable to future generations. The loss of genetic variation might further be considered a negative feature because, overall, the population involved faces reduced viability in the short term and loss of potential adaptability to changing environmental conditions in the long term (Soulé and Wilcox 1980, Allendorf and Leary 1986).

Allendorf and Leary (1986) and Scribner (1991) have shown that the loss of heterozygosity is of great concern for population conservation and that the health of many populations is deteriorating for this reason, whereas Lande (1988) focused the attention on demographic stochasticity, which could be of more concern than genetic problems.

Two main methods exist for the maintenance of genetic variation in domestic livestock. The first is the maintenance of native breeds that contain a well diversified set of characteristics and whose reproduction can be controlled (Maijala et al. 1984; Henson 1992); the second is the conservation of feral populations (domesticated animals that have returned to the wild state). According to Van Vuren and Hedrick (1989), feral populations may have two main categories of attributes which make them desirable for conservation: «First, feral populations may have relict characteristics or genetic variants that either are absent in modern breeds (...) Second, feral populations may have novel or rare characteristics or adaptations. These traits may include adaptations to extreme environmental conditions such as temperature stress, drought, high parasite load, or other characters of potential commercial or scientific value».

The horse *Equus caballus* has become extinct as a wild species, but feral horses exist in many places around the world, where they show great adaptability to different environmental conditions, ranging from the arid plains of Australia to the cold mountain ranges of western North America (Berger 1986). In Europe there are no feral horses, but in a few areas free roaming populations exist, managed by man to different degrees, eg. the New Forest (Tyler 1972) and Exmoor (Gates 1979) in England, the Camargue, France (Wells and von Goldschmidt-Rothschild 1979) and the Bialowieza forest and the Popielno reserve, Poland (Kownacki 1984).

Feral horses existed in Sardinia, Italy, at least as late as the end of the eighteenth century, when Cetti (1774) described the populations of the Island of Sant'Antioco and of the Nurra, the region at the North-western corner of Sardinia. These feral populations have not survived, and there are no completely feral horses. However many free ranging ponies live under moderate human interference in an area of central Sardinia, the Giara di Gésturi. The presence of ponies on the Giara can only be documented since the nineteenth century, but it is possible that a free ranging population existed there in earlier times (Cancedda, pers. comm.). These horses are privately owned by the inhabitants of towns surrounding the Plateau.

Giara ponies are well adapted to a difficult environment, characterized by drought, high temperature and food shortage during summer. The maintenance of a viable population of these ponies is desirable because they have been selected by an extreme Mediterranean climate, and thus can be a source of rustic characteristics for breeding purposes, for instance for the establishment of new controlled herds in formerly cultivated and recently abandoned agro-ecosystems in the Mediterranea area. The Giara ponies are at present of great economic, cultural and aesthetic value, and for the Sardinian people, as well as for other Italians, they are also an important part of our heritage (Ruiu 1988). Despite the fame of the Giara ponies in Italy, no data are available on their number, population structure or the management problems they are faced with.

In about 1976, a group of Giara ponies (2 males and 3 females) was translocated to a 12 km^2 protected area, managed by the Forest Service in the Capo Caccia peninsula, in North-western Sardinia, and gave origin to a naturally structured population.

In this paper an estimate of the population size in the Giara is given, as well as a description of the population structure and some aspects of the social behaviour of Giara ponies as compared with the ponies of Capo Caccia, focusing on the conservation problems of the Giara population under the current management regime and particularly on loss of heterozygosity as an indicator of genetic viability (Allendorf and Leary 1986).

Study area

The Giara di Gèsturi is a 45 km^2 plateau of volcanic origin with a sharp edge, dominating the intensively cultivated Marmilla region. Altitude varies between 493 and 609 m a.s.l. The soil is thin but very fertile and allows the growth of good pasture; some areas are not however covered by soil and the basaltic rock is exposed. About 46% of the plateau is covered by oak woods, where the prevailing tree species in the cork oak *Quercus suber*. The woods are characterized by a low canopy cover value: only 18% of the total surface area being occupied by wood whose canopy cover is at least 60%. 32% of the area is covered by various types of Mediterranean macchia, 10% by grassland, 9% by garrigue and 3% by temporary ponds (De Martis and Mossa 1988).

The area has a Mediterranean, mesomediterranean subregion, climate (Tomaselli et al. 1973) characterized by 759 mm of rain, mainly concentrated in autumn and winter and mean annual temperature of 15°. Generally speaking, this area experience a period of drought in summer and early autumn of about 4 months, whose effects are enhanced by frequent winds. This affects the growth of new pasture but does not prevent horses from drinking since water remains in the deepest ponds; furthermore there are a few permanent springs in the Giara.

There are some hundred free ranging cattle and goats which compete for food with horses. The plateau is uninhabited by man, apart from a few shepherd's huts. A recently built road enables vehicles to reach and cross the plateau, and in the summer months an increasing number of tourists visit the area.

Capo Caccia is a calcareous peninsula, mainly covered by garrigue and Mediterranean macchia, with some recent pine plantations.

Methods

Census

The census at Giara was performed by counting of all the horses in 3 large plots of known surface area (14.3 km^2 overall) and extrapolating the result to the total area. In all, 31.8% of the plateau was surveyed (Tab. 1). This is well over 10%, which is assumed to be the minimum sample which allows the extrapolation of census results to total area (Fattorini 1992). Preliminary observations indicated that herds were quite regularly spaced through the Giara, so that estimation in sample areas was feasible; indeed the coefficient of variation of density in the areas sampled was relatively low (14%). However, since the census plots were of unequal size and not chosen at random, it was impossible to calculate the confidence interval of the estimate (Krebs 1989). Recognizable individuals were repeatedly observed around the same spots at each visit, indicating that they did not move from one sample area to the other. In areas A and B (fig. 1), two observers, in mutual visual contact, thoroughly surveyed the sampled area by foot and counted the ponies they encountered. Double counts of the same individuals or bands were avoided by accurately describing the physical features of each horse. In C, horse bands were located at sunrise from the top of Mt. Zeppara Manna. Within three hours, each band was then approached by two observers, and an accurate count of individuals was effected.

Observations on the social structure were carried out during 5 visits to the Giara, from 28th September 1989 to 21st June 1990, for a total of 12 days of field work. The area was walked by foot, and the sex, age and a detailed description of physical features was registered for every individual observed, as well as the type of social unit. Much care was taken to ascertain the mother of foals and yearlings, as could be observed from mutual interactions (Tyler 1972, Wells and von Goldschmidt-Rothschild 1979). In all 520 individuals were observed; some individuals may have been encountered more than once during different visits, but this would not have caused a bias in the estimated population structure, as the resighting probability was the same for all age and sex classes.

Tab. 1 — Census results for the sample areas on the Giara di Gèsturi.

plot	area (km^2)	no. ponies	density (inds. / km^2)
A	3	40	13.3
B	2.8	29	10.4
C	8.5	89	10.5
Total	14.3	158	11.4

Fig. 1 — Map of the Giara di Gèsturi with census areas (letters). Thick line indicates the margin of the plateau. Thin line show ponds. Lower left figure indicates the position of the Giara di Gèsturi (dot) and of Capo Caccia (arrow) on Sardinia.

On Capo Caccia all the horses were individually known, as they were the object of intensive field work (Bogliani, in press).

Data on population structure were tested for homogeneity using the G-test for goodness of fit with Williams' correction (Sokal and Rohlf 1981).

Analysis

Effective population size and heterozygosity

The main feature of population structure in the Giara was unequal numbers of adult males and females. In this case the most suitable formula to calculate the Effective Population Size (N_e) (Wright 1969) is:

$$N_e = 4M*F/(M + F) \tag{1}$$

where M = number of males
F = number of females

Other population parameters, such as variance in progeny (males or females), unequal numbers in successive generations and non-random distribution of family size should be accounted for to have a thorough estimation of N_e (Falconer 1989), but these data are unavailable for the Giara or for any other feral population. Harris and Allendorf (1989) state that formula (1) might produce a large overestimation of N_e. However the estimate made with (1) can be used bearing in mind that N_e is probably small than calculated.

The loss of heterozygosity was calculated following Wright (1969) as:

$$H_t = H_0 \ (1 - 1/(2N_e))^t \qquad (2)$$

where t = time in generations
H_0 = initial heterozygosity (assumed to be 1)

Estimation of generation length

Feral and free ranging horses can live more than 20 years, while the first reproduction is usually at 3 years for females. This produces a great overlap between generations, and mean generation length could therefore be calculated from the net maternity function $l_x\ m_x$, which is the product of the fecundity m at the age x and the probability of survival l to the age x during the fertile period of adult females (Eberhardt 1985). Neither lx or mx were available for Giara ponies, however data on fecundity are available for 5 North American populations (Sable Island - Welsh 1975, in Berger 1986; Assateague Island - Keiper and Houpt 1984, in Berger 1986; Montana, free ranging - Speelman, Dawson and Phillips 1943, in Berger 1986; Great Basin - Berger 1986; Montana, feral - Garrott and Taylor 1990) and data on survival are given by Garrott and Taylor (1990). The medians of $l_x\ m_x$ of females during their fertile period for the above mentioned populations are 6, 7, 6 and 8, respectively. It is therefore reasonable to assume that the mean generation of Giara ponies is within the range of 6-8 years.

Results

Population size

On the Giara the extrapolation of the calculated mean density at the three census areas (11.4 individuals per km^2; Tab. 1) led to an estimated population size of 513 individuals. On Capo Caccia 25 individuals were present, with a density of 2.1 per km^2.

Population structure

The estimated sex ratio did not change through the study period ($G = 0.02$, $df = 3$, $n = 230$, n.s.), with an average of 0.12 males per female, or 1 male every 8.17 females, and a proportion of males among adults of 0.11.

The number of foals per female and of yearlings per female changed over the study period (Tab. 2). The relative number of foals was higher in autumn and winter and declined in spring, increasing during summer ($G = 39.9$, $df = 3$, $n = 102$, $P < 0.001$). Births mainly took place in spring, and to a lesser extent in summer. After April most young were classified as yearlings.

The peak in number of yearlings recorded was reached during summer, following the presumably normal rhythm of birth in previous year, then the number sharply declined ($G = 74$, $df = 3$, $n = 80$, $P < 0.001$) following the capture of a large number by pony owners. Most captured yearlings were removed and sold, but a small fraction of females was released after branding. A few individuals escaped capture.

On Capo Caccia, in spring and summer 1991, ponies were subdivided into five bands composed of one stallion and one or more mares with foals

Tab. 2 – Observations on population structure on the Giara di Gèsturi in 1989-90.

date	28-29 Sept.	12-14 Dec.	28 Feb.-2 Mar.	14 Apr.	19-21 Jun.	Total
males (adults)	5	–	7	2	10	24
females (adults)	44	64	54	17	81	260(196)*
males/females (adults)	1/8.8	–	1/7.71	1/8.5	1/8.1	1/8.17
foals	30	39	54**	4	29	102
yearlings	6	7		10	57	80
foals/females	0.68	0.61	–	0.23	0.36	0.49***
yearlings/females	0.14	0.11	–	0.59	0.70	0.39***

* the figure in parentheses indicates the number of females excluding those for December when males were not censused.
** foals and yearlings were not distinguishable.
*** these ratios were calculated excluding data for February-March.

and yearlings; there were also five bachelors. These horses are left unmanaged as regards the sex ratio and the social structure. The sex ratio of the population was close to one, but if only stallions holding a harem are considered, the ratio was 0.5.

Loss of heterozygosity

The Effective Population Size calculated, Ne, equals the adult population size when the sex ratio is 1 : 1, but can be very low when there is an unbalanced sex ratio (fig. 2). The estimated N_e of the Giara, calculated using M = 56 and F = 457 in formula (1), is $N_e = 199$.

The loss of heterozygosity, calculated with formula (2), is shown in fig. 3. If the current sex ratio management is continued in the future, a 10% loss of heterozygosity may be expected in 42 generations (294 years). 50% of heterozygosity will be lost in 276 generations (1932 years).

Discussion

Comparison of the population structure of the ponies in the Giara with that of Capo Caccia, whose sex ratio and age ratio are not altered by man, shows some differences (Tab. 3). There is some evidence in feral horses that the proportion of males siring foals is higher than might be expected if only harem stallions have access to fertile females (Bowling and Touchberry 1990); in fact some adult bachelors or low-rank males in multi-male bands are likely to copulate with oestrous females. Therefore the real sex ratio in Capo Caccia is probably closer to 1 : 1. In The Giara the sex ratio is heavily altered by man, and almost all yearling males are removed. During our observations, only 3 yearling and 1 two-year-old males were observed after the autumn round-up by horse owners. It seems unlikely that any foal be sired by very young males (Berger 1986), so that the observed sex ratio is reliable.

The number of foals per year per female was the same in both areas (0.49-0.50), and was consistent with birth-rate figures for North American feral populations: Assateague Island, 0.57 (Keiper and Houpt 1984, in Berger 1986), Sable Island, 0.60 (Welsh 1975, in Berger 1986), Great Basin, 0.54-0.60 (Berger 1986), Montana 0.49 (Garrott and Taylor 1990).

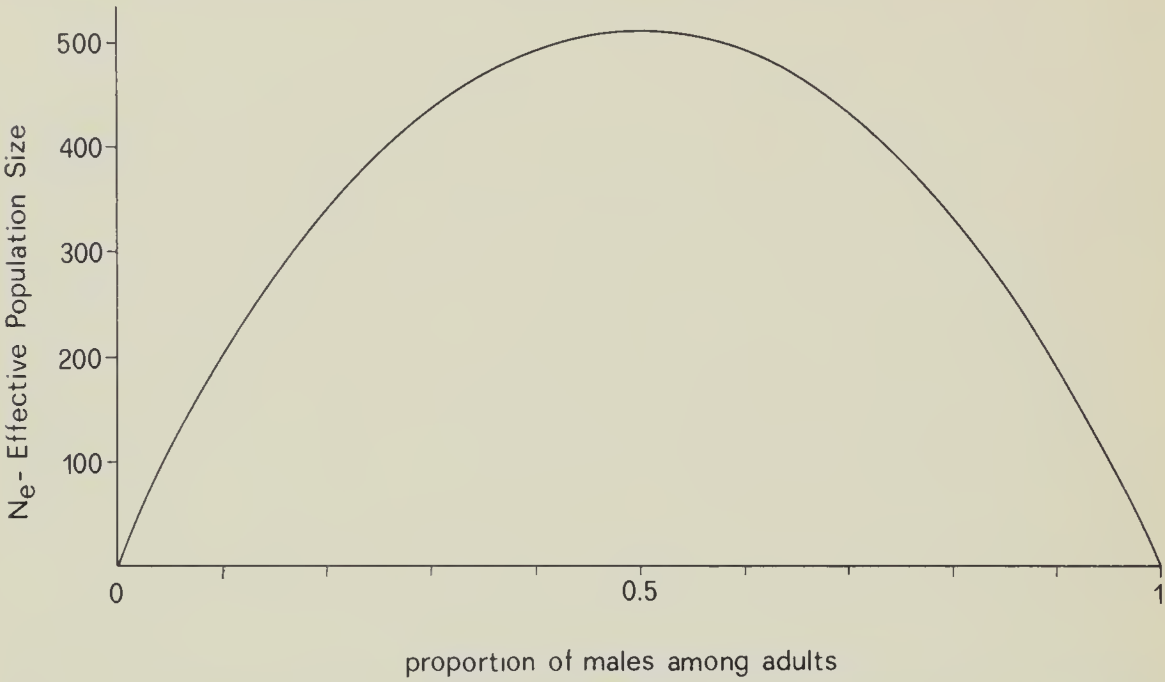

Fig. 2 — The Genetically Effective Population Size as a function of the proportion of males in the reproductive population of Giara ponies.

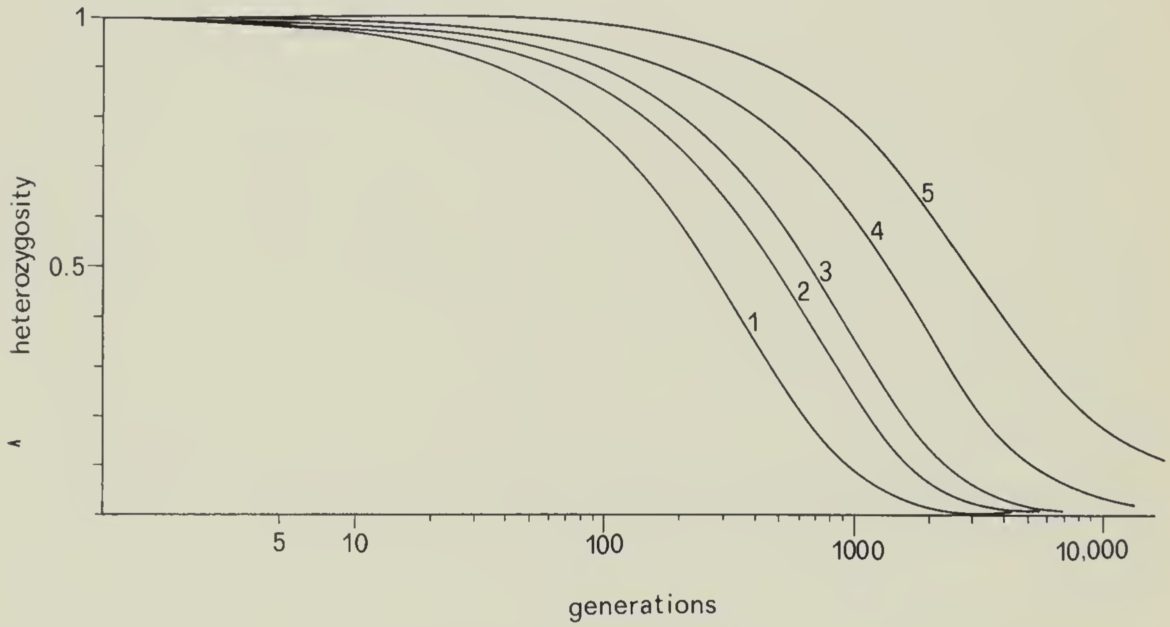

Fig. 3 — Scenario of variation of heterozygosity (assuming the present value of H_0 to be one) through time with different Effective Population Sizes on the Giara.

1 - N = 513 (sex-ratio = 1 male/8.17 females)
N_e = 199
2 - N = 1000 (sex-ratio = 1 male/8.17 females)
3 - N_e = N = 513 (sex-ratio = 1 male/1 female)
4 - N_e = 1000
5 - N_e = 2000

Tab. 3 – Features of free ranging pony populations in Sardinia.

	Giara di Gèsturi	Capo Caccia
population size	513	27
males/females (adults)	1/8.17	1/0.91 (1/2)*
foals/females	0.49	0.50
yearlings/females	0.70-0.11	0.57

* the figure in parentheses refers to males owning a harem.

There are two mechanisms which counteract the effect of a small N_e in free ranging horses. The first is natural selection, by which individuals carrying deleterious homozygote alleles should show a lowered fitness. The second mechanism is the tendency to exogamy, which results in a non-random choice of mating partners (Duncan et al. 1984).

In captive propagation schemes a common goal is the maintenance of 90% of the average heterozygosity for 200 years (Soulè 1986). These values are arbitrary, and were chosen in the belief that, within this timespan, human population will have stabilized is growth and new technologies will be of help in maintaining genetic diversity. The Giara ponies face a serious threat of the rapid loss of genetic variability especially if minor changes in the wrong direction occur. Two alternative measures may be taken in order to reduce the loss of heterozygosity. The first is the maintenance of the present total population size, but with a sex ratio of 1, which would maximize the effective population size. Under this regime, 10% of heterozygosity would be lost in 105 generations (735 years) and 50% would be lost in 693 generations (4851 years). These values are more than twice as high as with the current unbalanced sex ratio.

An alternative measure to reduce the loss of heterozygosity would be an increase in numbers (N). With 1000 individuals and the current sex ratio, the estimated Ne would be 392. In this case, 10% of heterozygosity would be lost in 82 generations (574 years) and 50% in 543 generations (3801); these values are lower than those achievable where $N = N_e = 513$, which is the maximum estimated N in the case of perfectly balanced sex ratio. With a sex ratio of 1 : 1 and $N = N_e = 1000$, 10% of heterozygosity would be lost in 228 generations (1596 years) and 50% in 1505 generations (10,536 years). With $N = 2000$ and a sex ratio of 1 : 8.17, N_e would be 748; in this case 10% of heterozygosity would be lost in 165 generations (1155 years) and 50% in 1086 generations (7602 years). With a sex ratio of 1 : 1 and $N = N_e = 2000$, 10% of heterozygosity would be lost in 415 generations (2905 years) and 50% in 2736 generations (19,152 years).

In 200 years, or about 29 generations, at least 7% of heterozygosity will be lost under the current management regime. This figures are optimistic because of the probability of the overestimation of N_e discussed above. The goal of maintaining a large portion of the present level of heterozygosity for the future could be achieved 1) by maintaining the present population size unaltered and achieving a sex ratio closer to 1 : 1, or 2) by increasing the population size maintaining the current unbalanced sex ratio. Increasing the population size might cause a heavy impact on vegetation. The first solution seems to be more suitable but would mean lower income for horse owners because the number of fertile females will be reduced. This problem could be solved by means of aid to owners given by public agencies interested in wildlife management and conservation or in rare stock maintenance, such as the Sardinian Autonomous Regional Authority.

Manipulations which would mitigate natural mortality, such as supplementary feeding and veterinary help, should be discouraged because they could enhance the survival of individuals bearing recessive deleterious characteristics in homozygosity which could spread in the population as a result of genetic drift.

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